

CLAIMS

1. A biologically active lipid mobilising agent for use in therapy which has an apparent molecular mass  $M_r$  as determined by gel exclusion chromatography greater than 6.0 kDa, and which is capable of inducing lipolysis in mammalian adipocytes, characterised in that it has the properties and characteristics of a Zn- $\alpha_2$ -glycoprotein.
2. A purified biologically active lipid mobilising agent as claimed in Claim 1 for use in therapy characterised in that it is substantially free of proteolytic activity and consists essentially of a glycosylated polypeptide having an apparent relative molecular mass  $M_r$  of about 43kDa as determined by its electrophoretic mobility when subjected to 15% SDS-PAGE electrophoresis and having homology in amino acid sequence with the amino acid sequence (SEQ ID No: 1) of human plasma Zn- $\alpha_2$ -glycoprotein.
3. A lipid mobilising agent as claimed in Claim 2 further characterised in that it is obtainable by a process that includes sequential steps of subjecting biological material to ion exchange chromatography, exclusion chromatography, and then to hydrophobic interaction chromatography, said biological material being urine from a cancer cachexia patient or an extract of a culture of a MAC16 tumour cell line deposited under the provisions of the Budapest Treaty in the European Collection Of Animal Cell Cultures (ECACC) under an Accession No. 89030816.
4. A biologically active lipid mobilising agent as claimed in Claim 1 for therapeutic use which is a glycosylated polypeptide wherein the polypeptide moiety is selected from one of the following groups:
- a) a polypeptide having the amino acid sequence of a Zn- $\alpha_2$ -glycoprotein;
  - b) a polypeptide which in respect to (a) is deficient in one or more

amino acids that do not significantly affect the lipid mobilising or lipolytic activity;

c) a polypeptide in which in respect to (a) one or more amino acids are replaced by a different amino acid or acids that do not significantly affect the lipid mobilising or lipolytic activity;

d) a polypeptide in which in respect to (a) there is incorporated a plurality of additional amino acids which do not interfere with the biological lipolytic activity.

5. A biologically active lipid mobilising agent for use in therapy as claimed in Claim 1 consisting essentially of a glycoprotein that has a polypeptide amino acid sequence homologous with the amino acid sequence (SEQ ID No: 1) of human plasma Zn- $\alpha_2$ -glycoprotein, or with a variant thereof which is modified by minor additions, deletions, or substitutions that do not substantially affect its lipid mobilising activity in biological systems.

6. A lipid mobilising agent for use in therapy as claimed in Claim 4 or 5 further characterised in that it has an apparent relative molecular mass  $M_r$  of about 43kDa as determined by its electrophoretic mobility when subjected to 15% SDS-PAGE electrophoresis.

7. A lipid mobilising agent for use in therapy as claimed in any one of Claims 1 to 6 further characterised in that when subjected to digestion with chymotrypsin its lipid mobilising properties are destroyed.

8. A lipid mobilising agent for use in therapy as claimed in any one of Claims 1 to 7 further characterised in that it has the potential *in vitro* to stimulate adenylate cyclase activity in a guanine triphosphate (GTP) dependent process upon incubation with murine adipocyte plasma membranes.

9. A lipid mobilising agent for use in therapy as claimed in any one of Claims 1 to 8 further characterised in that it has substantially the same

immunological properties as human Zn- $\alpha_2$ -glycoprotein.

10. A biologically active lipid mobilising agent for use in therapy which is capable of inducing lipolysis in mammalian adipocytes characterised in that it has an apparent molecular mass  $M_r$  as determined by gel exclusion chromatograph greater than 6.0kDa and is obtainable by subjecting the lipid mobilising agent claimed in any one of the preceding claims to fragmentation by enzymatic degradation.

11. A biologically active lipid mobilising agent as claimed in Claim 10 for use in therapy that is a fragment of a glycoprotein or glycosylated polypeptide which is a component of the lipid mobilising agent claimed in any one of Claims 1 to 9 produced by digesting the latter with trypsin

12. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that it is substantially free of proteolytic activity.

13. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that the polypeptide chain of the polypeptide component has an N-terminus blocked by a pyroglutamate residue.

14. A lipid mobilising agent for use in therapy as claimed in any one of the preceding claims further characterised in that the lipid mobilising activity is destroyed by periodate treatment.

15. Use of a lipid mobilising agent as claimed in any of the preceding claims for the manufacture of a medicament useful in human medicine for treating conditions of overweight or obesity and/or for stimulating muscle development.

16. A method of isolating and purifying a lipid mobilising agent having the properties and characteristics of a Zn- $\alpha_2$ -glycoprotein, said method comprising subjecting an extract of a cachexia-inducing tumour or of a culture of a

cachexia-inducing tumour cell line, or a sample of urine or other body fluid of a mammal bearing a cachexia-inducing tumour, to a combination of ion exchange, gel filtration size exclusion chromatography, and hydrophobic interaction chromatography, and recovering a single product or molecular species having an apparent relative molecular mass of 43kDa, as determined by 15% SDS-PAGE electrophoresis, which is substantially free of proteolytic activity.

17. A pharmaceutical composition for use in treating mammals, said composition containing as the active constituent an effective therapeutic amount of a lipid mobilising agent as claimed in any one of Claims 1 to 14, together with a pharmaceutically acceptable carrier, diluent or excipient.

18. A pharmaceutical composition as claimed in Claim 17 which is an injectable formulation incorporating a carrier in the form of a pharmaceutically acceptable injection vehicle.

19. A method of treating a mammal to bring about a weight reduction or reduction in obesity, said method comprising administering to the mammal in need of such treatment a therapeutically effective dosage of a lipid mobilising agent as claimed in any one of Claims 1 to 14.

20. A method of treating a mammal to bring about a weight reduction or reduction in obesity, said method comprising administering to the mammal in need of such treatment a therapeutically effective dosage of a glycoprotein identical to or homologous with human Zn- $\alpha$ 2-glycoprotein, or an effective lipolytically active fragment thereof which has an apparent molecular mass Mr as determined by gel exclusion chromatography that is greater than 6.0kDa, substantially free of any proteolytic activity.

21. A diagnostic method for detecting the presence of a tumour in a mammal and/or for monitoring the progress of treatment of such a tumour, said

method comprising taking from said mammal a sample of urine, blood serum or other body fluid and testing to detect the presence of and/or to measure the amount therein of Zn- $\alpha_2$ -glycoprotein.

22. A diagnostic method as claimed in Claim 21 wherein the testing is carried out by use of a biochemical reagent capable of specifically recognising and binding to Zn- $\alpha_2$ -glycoprotein.

23. A diagnostic method as claimed in Claim 22 wherein the biochemical reagent is a monoclonal or polyclonal antibody.

24. A diagnostic method as claimed in any one of Claims 21 to 23 further characterised in that it is applied to a sample of urine.

25. A diagnostic kit for carrying out the method of Claim 21 or 22, said kit comprising a receptacle for receiving the sample of body fluid, a biochemical reagent for detecting Zn- $\alpha_2$ -glycoprotein, and instructions for use of said kit.

26. Use of a lipid mobilising agent as defined in any one of Claims 1 to 14 for producing antibodies for use as a diagnostic detecting agent for use in therapy as inhibitors or antagonists to the lipid mobilising agent causing cachexia in cancer patients.

27. Use of a preparation of antibodies for the manufacture of a medical preparation or medicament for the treatment of cachexia-associated cancer and/or tumours, wherein said antibodies are capable of specifically recognising and binding to the lipid mobilising agent claimed in any one of Claims 1 to 14.

28. Use as claimed in Claim 27 of a preparation of antibodies wherein the antibodies are monoclonal antibodies.

29. Use of a lipid mobilising agent as defined in any one of Claims 1 to 14 for screening and identifying and/or for carrying out investigations of possible lipolytic activity inhibiting agents having potential as anti-cachectic or antitumour therapeutic agents.

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